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14. ABSTRACT

The hypothesis being tested in this project is that a greater proportion of African-Americans with breast cancer harbor a specific germline genetic alteration in the ATM gene or possess a particular ATM haplotype, compared to African-American women without breast cancer. An additional objective is to determine the functional impact upon the protein encoded by the ATM gene for each mutation identified.

Specific Aims: The specific aims of this project are to (1) screen 100 African-American breast cancer patients and 100 African-American women without breast cancer and (2) perform functional studies using cells from patients identified as ATM carriers to determine whether each ATM genetic variant identified affects radiosensitivity and levels of the protein encoded by the ATM gene for each mutation examined.

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Introduction

African-American women on average present with more advanced breast cancer when compared with Caucasian women. This leads to suboptimal cure rates within this population. Numerous investigators have attempted to explain this discrepancy and determine if it stems from an inherent aggressive biologic behavior or a lack of access to appropriate medical care. It is still controversial, but there is evidence from randomized trials that when controlled for stage, African-American women have similar outcomes compared with their ethnic counterparts. However, it appears that socioeconomic factors lead to delayed screening evaluations and disease that is locally or systemically advanced rather than pre-emptive identification of early stage breast cancer. It would therefore be useful to discover a genetic marker that can serve to identify African-American women who are at increased risk for breast cancer at an age prior to disease development. The *ATM* gene has been chosen for consideration as a potential marker given its critical role in the maintenance of genomic integrity.

The hypothesis to be tested in this project is that a greater proportion of African-Americans with breast cancer harbor a genetic alteration in the *ATM* gene compared to African-American women without breast cancer. An additional objective is to determine the functional impact upon the protein encoded by the *ATM* gene for each mutation identified.

The specific aims of this project are to (1) screen 100 African-American breast cancer patients (cases) and 100 African-American women without breast cancer (controls) and (2) perform functional studies using cells from patients identified as *ATM* carriers to determine whether each *ATM* variant identified affects radiosensitivity and levels of the protein encoded by the *ATM* gene for mutations identified.

Body

STATEMENT OF WORK

Screening for *ATM* Mutations in an African-American Population to Identify a Predictor of Breast Cancer Susceptibility

Task 1 (Months 1-30): Identify 100 newly diagnosed African-American breast cancer patients (cases) seen in the Radiation Oncology departments at Mount Sinai and NYU for oncologic consultation who are eligible for participation using the criteria outlined in the proposal and obtain written informed consent and blood samples.

Task 2 (Months 1-30): Identify 100 African-American women (controls) when they are screened by the Mount Sinai Breast Imaging Service and found to be free from breast cancer and obtain written informed consent and blood samples.

Task 3 (Months 1-30): Isolate DNA from blood lymphocytes of patients.

Task 4 (Months 1-36): Perform PCRs with these samples to amplify each exon of the *ATM* gene.

Task 5 (Months 1-36): Use DHPLC to identify PCR products that may possess mutations based upon the appearance of aberrant chromatograms.

Task 6 (Months 3-36): Sequence all PCR products that display aberrant DHPLC chromatogram profiles.

Task 7 (Months 6-36): Determine ATM protein levels in lymphoblastoid cell lines.

Task 8 (Months 6-36): Determine radiosensitivity of lymphoblastoid cell lines

Task 9 (Months 6-36): Perform statistical analyses of the data generated in this project.

As described below, all nine tasks outlined in the approved Statement of Work have been accomplished.

As detailed in Table 1, a total of 54 unique *ATM* variants were detected either in one of the 62 coding exons of *ATM* or within 10 bases of the exon/inton boundaries in potential splice site regions for 51 of the 79 cases (65%) whereas 223 variants were found in 171 of the 244 controls (70%) ($p = 0.48$). Thus, there was not a statistically significant difference between the percentage of women who developed breast cancer that possessed an *ATM* variant compared with women who did not develop breast cancer. Furthermore, there was no significant difference in the frequency of any specific variant between the cases and controls. Hence, the results of this study do not support the hypothesis that *ATM* variants represent genetic factors that are either associated with or predictive for the development of breast cancer in African-American women.

However, with that said, an important discovery in this study was a dramatic difference between the types of genetic variants found in African-American subjects compared with non-African-Americans. Through a separate project, we screened 335 non-African-American subjects. Thus, we are in a position to compare our findings for African-Americans with a non-African-American cohort, the results of which are shown in Table 2. A total of 80 distinct genetic variants were identified. Of the genetic alterations detected, 29 were found to be unique in the non-African-American population while 33 were found only among African-Americans. Hence, only 18 of the variants were found to be in common for the two groups. Furthermore, of the eight variants identified in the non-African-American population that were present at a frequency greater than 2%, only the 5557G>A polymorphism, was also detected in more than 2% of African-Americans. However, as opposed to a rate of 25% among non–African-Americans, only 4% of the African-Americans exhibited this polymorphism. In addition, of the 8 variants found in more than 2% of African-Americans examined, other than the 5557G>A variant, none were present at a frequency greater than 1% among non-African-Americans. Of particular note is the 378T>A polymorphism, which was found in 25% of the African-American cohort, but only about 1% of the subjects outside of this group. These large differences in the types of variants detected and their relative frequency in the African-American compared with the non-African-American population, provide strong evidence that significant differences exist in the genetic profiles between these groups.

Table 1. Summary of Genetic Variants Identified in Cases and Controls

Polymorphism	AA change	Cases (N=79) (%)	Controls (N=244) (%)
IVS28+5 G→T	N/A	0 (0)	1 (0.4)
IVS25+5 T→C	N/A	0 (0)	1 (0.4)
IVS38-8 T→C	N/A	0 (0)	1 (0.4)
IVS5-7 C→T	N/A	0 (0)	3 (1)
IVS53-2 A→ G	N/A	0 (0)	1 (0.4)
320 G → A	C107Y	0 (0)	1 (0.4)
334 G → A	A112T	0 (0)	1 (0.4)
370 A → G	I124V	0 (0)	3 (1)
378 T → A	D126E	14 (18)	67 (27)
544 G→C	V182C	0 (0)	1 (0.4)
975 A→C	K1992T	0 (0)	1 (0.4)
1073 A→G	N358S	0 (0)	2 (1)
1176 C → G	G392G	3 (4)	13 (5)
1254 A → G	Q418Q	3 (4)	18 (7)
1541 G → A	G514D	1 (1)	7 (3)
1595 G → A	C532Y	0 (0)	1 (0.4)
1636 C→G	L546V	0 (0)	4
1744 T → C	F582L	0 (0)	1 (0.4)
1773 T→C	N591N	0 (0)	1 (0.4)
1810 C → T	P604S	1 (1)	3 (1)
2096 A → G	E699G	0 (0)	3 (1)
2289 T → A	F763L	2 (3)	2 (1)
2362 A → C	S788R	0 (0)	3 (1)
2442 C→A	D814E	1 (1)	0 (0)
2572 T → C	F828L	1 (1)	3 (1)
2610 C → T	N870N	0 (0)	1 (0.4)
2614 C → T	P872S	0 (0)	6 (2)
2685 A→ G	L895L	5 (6)	13 (5)
3161 C→G	P1054P	0 (0)	1 (0.4)
3383 A → G	Q1128P	4 (5)	11 (5)
4042 T → C	L1348C	0 (0)	5 (2)
4138 C→T	H1380Y	3 (4)	3 (1)1
4258 C→T	L1420F	0 (0)	1 (0.4)
4279 G → A	A1427T	1 (1)	0 (0)
4424 A → G	Y1475C	0 (0)	2 (1)
4400 A→G		1 (1)	0 (0)
4466 G→A	R1459H	0 (0)	1 (0.4)
4578 C→T	P1525P	2 (3)	4 (2)
4939 C → T	L1647L	1 (1)	0 (0)
5557 G → A	D1853N	7 (9)	6 (2)
5558 A→T	D1853V	1 (1)	1 (0.4)
5793 T → C	A1931A	1 (1)	5 (2)

6088 A→G	I2030V	0 (0)	1 (0.4)
6176 C → T	T2059I	0 (0)	2 (1)
6235 G → A	V2079I	1 (1)	6 (2)
6437 G→C	S2146T	0 (0)	3 (1)
6995 T → C	L2332P	0 (0)	1 (0.4)
7313 C → T	T2438F	0 (0)	1 (0.4)
8757 C → T	G2919G	0 (0)	2 (1)
9200 C→G	N/A	0 (0)	4 (2)
9215 A→G	N/A	1 (1)	1 (0.4)

Table 2. *ATM* genetic variants identified in non-African-Americans and African-Americans

VARIANT	PERCENTAGE NON-AFRICAN-AMERICAN (N=335)	PERCENTAGE AFRICAN-AMERICAN (N=323)
162T>C	0.3	0
198A>C	0.3	0
320G>A	0	0.3
334G>A	0	0.3
370A>G	0	0.9
378T>A	0.9	**25.2**
544G>C	0	0.3
735C>T	1.2	0
975A>C	0	0.3
993G>A	0.3	0
998C>T	0.3	0
1073A>G	0	0.6
1176C>G	0.3	**5.0**
1229T>C	0.6	0
1254A>G	0	**6.5**
1329A>C	0.3	0
1541G>A	0	**2.5**
1595G>A	0	0.3
1636C>G	0.3	1.2
1744T>C	0	0.3
1773 T→C	0	0.3
1810C>T	0.6	**2.1**
1986T>C	1.2	0
2038T>C	0.3	0
2085G>A	0.3	0
2091A>C	0.3	0
2096A>G	0	0.9
2119T>C	**2.1**	0
2289T>A	0	1.2

2362A>C	0.3	0.9
2442C>A	0.6	0.3
2572T>C	**2.7**	1.2
2610C>T	0	0.3
2614C>T	1.5	1.9
2685A>C	0.6	5.6
3118A>G	0.3	0
3161C>G	**2.4**	0.3
3383A>G	0.3	**4.7**
4042 T→C	0	1.6
4138C>T	0	1.9
4258C>T	**3.9**	0.3
4279G>A	0	0.3
4326T>C	0.3	0
4388T>G	0.9	0
4400A>G	0	0.3
4424 A→G	0	.9
4466 G→A	0	.3
4473C>T	0.6	0
4578C>T	**9**	1.9
4939C>T	0	0.3
5071A>C	0.3	0
5557G>A	**24.9**	**4.0**
5558A>T	1.8	.6
5793T>C	1.2	1.9
5975A>C	0.3	0
6088 A→G	0	.3
6176C>T	0	0.6
6235G>A	0	**2.2**
6437G>C	0	0.9
6658C>T	0.3	0
6919C>T	0.6	0
6995 T→C	0	0.3
7313C>T	0	0.3
7368del9	0.3	0
7889T>A	0.6	0
8071C>T	0.3	0
8757 C → T	0	0.6
9200C>G	0.6	1.2
9215A>G	0	0.6
IVSS10-6T>G	1.2	0
IVS21-8insT	0.6	0
IVS25+5 T→C	0	0.3

IVS28+5G>T	0	0.3
IVS37+7G>C	0.6	0
IVS38-8T>C	**2.1**	0.3
IVS5-7C>T	0	0.9
IVS53-2 A→G	0	0.3
IVS54+8G>T	0.9	0
IVS5-8T>G	0.3	0
IVS62+8A>C	**3**	0

In addition to screening for genetic alterations, a series of EBV lymphoblastoid cell lines were created with lymphocytes derived from the subjects enrolled in this study. For experiments in which p53 phosphorylation was measured, cells were irradiated with either 0 or 4 Gy of x-rays and incubated either 0.5 or 2 hr. The densitometric results for each time point were divided by the value in each experiment for unirradiated cells to normalize these results. Each irradiation was performed a total of three times. The mean values (with standard deviations) for five wild type cell lines incubated either 0.5 or 2.0 hr were 3.6+1.4 and 6.5+2.3, respectively. The results for the cell lines possessing variants are shown in Table 3. ATM protein levels were also measured in each cell line irradiated in three separate experiments and divided by the average value obtained for the five wild type *ATM* cell lines. The same irradiations were performed with a series of 6 ATM heterozygote cell lines derived from parents of people diagnosed with ataxia telangiectasia and 8 ATM homozygote cell lines derived from people with ataxia telangiecatasia.

Although some variation was found, the only statistically significant results in these studies were obtained for the ATM levels in *ATM* homozygote cell lines derived from people diagnosed with ataxia telangiectasia. The levels of phosphorylated p53 were also generally lower in the *ATM* homozygote cell lines compared with wild type. In addition, the *ATM* heterozygote cell lines displayed somewhat lower levels of both ATM and phosphorylated p53 compared with wild type. However, the levels of ATM and phosphorylated p53 varied for the cases and controls screened in this project with no general pattern. Thus, the presence of an *ATM* variant does not necessarily result in an altered level of ATM protein produced or its activity. Hence, no evidence was obtained in this study that the *ATM* missense variants that are commonly detected have a dominant negative effect upon ATM protein function that would yield functional results similar to those obtained for people diagnosed with AT.

The radiosensitivity of each cell line was also determined from the growth response of cells irradiated with either 0, 0.5, 1.0 or 2.0 Gy of X-rays by extrapolating the growth curve to the intercept at zero time. The radiosensitivity of each cell lines was estimated from the α-value ($S = e^{-\alpha D}$) normalized to the value obtained for wild type cells listed in Tables 3 and 4. The α-values for the cell lines derived from AT patients were all significantly greater than one. In addition, the α-values for the AT heterozygotes were consistently greater than one, although generally not significantly greater. In contrast, the α-values for the cell lines obtained from the breast cancer patients were variable and none was significantly greater than one.

Table 3. Functional Assays of Lymphoblastoid Cells Derived from Subjects Possessing *ATM* Variants

Cell Line	Case or Control	Nucleotide Change	Amino Acid Substitution	ATM level	Phospho-p53 0.5 hr	Phospho-p53 2 hr	Normal-ized α-value
MS01-83	Control	378 T>A 1176 C>G	126 D>E 392 G>G	1.1±0.4	1.1±0.1	1.8±0.8	1.2±0.2
MS02-27	Control	378 T>A 1254 A>G 2289 T>A	126D>E 418Q>Q 763 F>L	1.0±0.4	2.1±0.8	1.6±0.8	1.0±0.1
MS02-47	Control	1541 G>A	514 G>D	0.9±0.3	3.4±3.4	6.3±5.6	1.4±0.4
MS02-57	Control	2614 C>T 2685 A>G	872 P>S 895L>L	1.6±0.4	3.3±3.60	2.3±0.8	0.8±0.3
MS02-70	Control	378 T>A 6437 G>C 9215 A>G	126 D>E 2146 S>T	0.8±0.5	2.7±1.6	8.3±3.8	1.4±0.3
MS02-71	Control	378 T>A 2096 A>G	126 D>E 699 E>G	1.3±0.7	2.2±2.6	5.0±4.6	1.1±0.1
MS03-10	Control	378 T>A 6176 C>T	126 D>E 2059 T>I	1.0±0.3	1.8±0.4	8.3±3.2	1.0±0.2
MS03-39	Control	975 A>C IVS62+8 A>C	1992 K>T n/a	0.7±0.3	1.6±1.6	3.0±3.2	0.8±0.2
MS03-46	Control	378 T>A 544 G>C	126 D>E 182 V>C	0.5±0.2	1.2±0.3	2.4±1.5	1.0±0.2
MS01-33	Case	4138 C>T	1380 H>Y	1.1±0.6	5.1±4.4	5.4±3.0	1.2±0.2
MS01-30	Case	IVS5-7 C>T	N/A	0.5±0.3	2.0±1.7	4.5±4.0	1.0±0.4
MS01-39	Case	5557 G>A 5558 A>T	1853 D>N 1853 D>V	1.3±0.9	1.4±1.0	2.7±0.4	1.1±0.5
MS01-45	Case	5557 G>A	1853 D>N	0.4±.04	1.4±.0.6	1.6±1.0	1.3±0.1
MS01-51	Case	IVS5-7 C>T	N/A	0.7±0.5	2.5±2.6	9.5±4.5	0.5±.2
MS01-37	Case	378 T>A 1176 C>G 4138 C>T	126 D>E 392 G>G 1380 H>Y	1.6±0.2	2.1±1.2	2.0±1.2	1.4±0.4
MS01-67	Case	4578 C>T	1526 P>P	0.5±.09	4.6±0.8	10.7±3.7	1.2±0.1
MS01-65	Case	5557 G>A	1853 D>N	1.1±0.5	2.7±1.2	4.1±2.0	1.2±0.3
Ms01-53	Case	378 T>A 1176 C>G	126 D>E 392 G>G	1.0±0.1	2.5±0.8	6.5±2.1	0.8±0.3

Table 4. Functional Assays of *ATM* Homozygoyte and *ATM* Heterozygote Lymphoblastoid Cell Lines

Cell Line	Homozygote or Heterozygote	ATM Level	Phospho-p53 0.5 hr	Phospho-p53 2 hr	Normalized α-value
8388	heterozygote	0.7±0.6	1.6±0.2	6.7±2.3	1.5±0.2
8925	heterozygote	0.7±0.8	1.9±0.4	5.1±0.1	1.4±0.2
8928	heterozygote	0.8±0.3	3.8±3.5	3.5±2.7	1.7±0.2
9579	heterozygote	0.5±0.3	2.3±1.3	2.6±0.3	1.1±0.3
2781	heterozygote	0.7±0.5	3.2±0.6	4.5±4.1	1.6±0.2
9588	heterozygote	0.5±0.5	6.1±4.0	6.9±2.8	1.2±0.3
8436	homozygote	0.04±0.06	2.9±1.2	2.8±0.4	1.8±0.3
9581	homozygote	0.08±0.02	1.5±1.7	4.0±1.6	2.0±0.3
9582	homozygote	0.05±0.02	2.0±4.4	2.1±0.4	2.2±0.3
2782	homozygote	0.08±0.05	2.1±3.1	3.1±1.3	2.1±0.3
1525	homozygote	0.05±0.02	2.6±1.1	3.1±1.2	1.8±0.2
11254	homozygote	0.09±0.06	1.8±0.1	2.5±0.9	2.3±0.3
9586	homozygote	0.24±0.22	1.7±1.0	4.3±1.9	1.8±0.4
13328	homozygote	0.13±0.09	0.6±0.5	2.1±1.3	2.1±0.3

Key Research Accomplishments

- The overall percentage of *ATM* variants did not differ significantly between African-American women who have been diagnosed with breast cancer (cases) compared with an age-matched group of African-American women (controls) who were not diagnosed with breast cancer.
- The percentage of any specific *ATM* variant did not differ significantly between cases and controls.
- A substantial and remarkable difference was detected in the types of *ATM* variants identified in African-Americans compared with a non-African-American cohort.
- No significant difference was found in any of the functional studies performed between cases and controls.

Reportable Outcomes

A paper reporting the results of this work is currently being prepared.

Conclusions

The results of this project do not support the hypothesis that a greater proportion of African-American women diagnosed with breast cancer harbor a specific genetic alteration in the *ATM* gene, or possess a particular *ATM* haplotype, compared to African-American women without breast cancer. However, an important finding of this study is that there is a surprising lack of overlap between the *ATM* genetic variants detected in African-Americans compared with a non-African-American population. This finding has critical implications for the numerous genetic studies being performed in which efforts are being made to link specific genetic variants with disease susceptibility. Although it appears from the results of this study that genetic variation in *ATM* is not associated with breast cancer susceptibility for African-American women, the results of this work strongly argue for the importance of including substantial numbers of African-Americans in all genetic studies being performed as it is likely that the results obtained for non-African-American populations are not applicable to African-Americans.

References

None

Appendices

None